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FIG. 1



FIGURE 1

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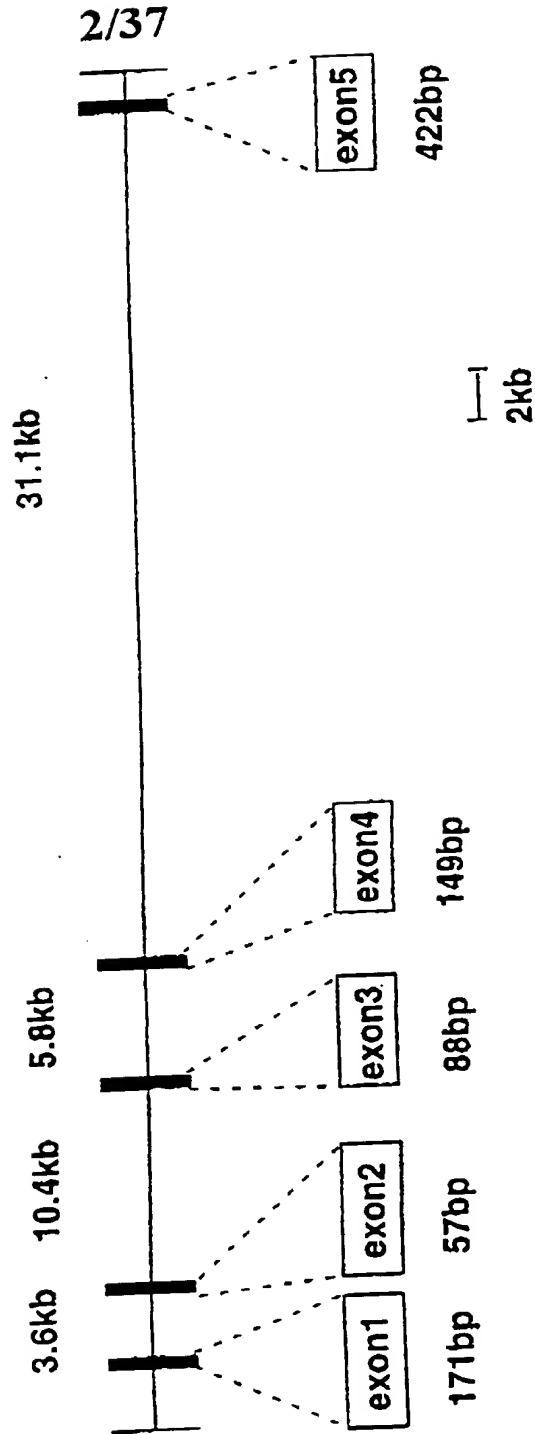


FIGURE 2

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Human Csl M S K Q P V S N V R A I Q A N I N I P N G A F R P G A G Q P P
 Mouse Csl M S K Q P I S N V R A I Q A N I N I P M G A F R P G A G Q P P
 Xenopus Csl M S K Q P A S N I R S I Q A N I N I P M G A F R P G A G Q P P

Human Csl R R K E C T P E V E E G V P P T - S D - - - E E K K P I P G
 Mouse Csl R R K E S T P E T E E G A P T T - S - - - E E K K P I P G
 Xenopus Csl K R K E F S T E E - E Q H V P T P E S E E K S E E K K P I P G

Human Csl A K K L P G P A V N L S E I Q N I K S E L K Y V P K A E Q .
 Mouse Csl M K K F P G P V V N L S E I Q N V K S E L K F V P K G E Q .
 Xenopus Csl A V K L P G P A F N L S E T .

FIGURE 3

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L.intestine
 Uterus
 Bladder
 Sk.muscle
 S.intestine
 Kidney
 Pancreas
 Testes
 Ovary
 Spleen
 Stomach
 Liver
 Lung
 Heart
 Thymus
 S.gland
 Brain
 Tongue
 Skull

Csl

Cyclo

FIGURE 4

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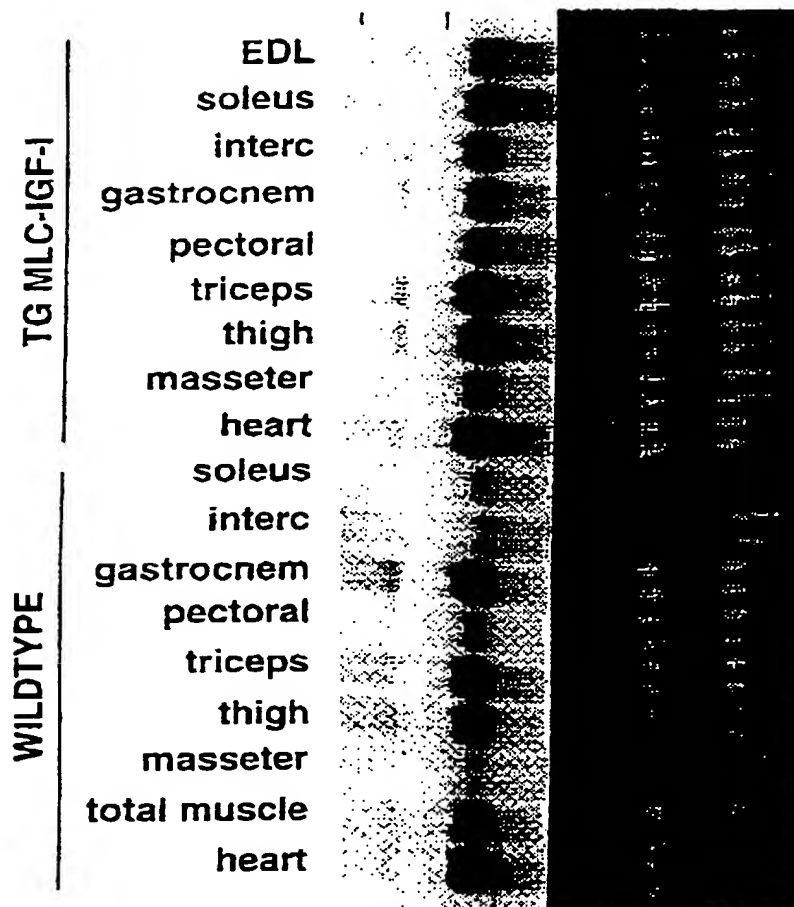


FIGURE 5

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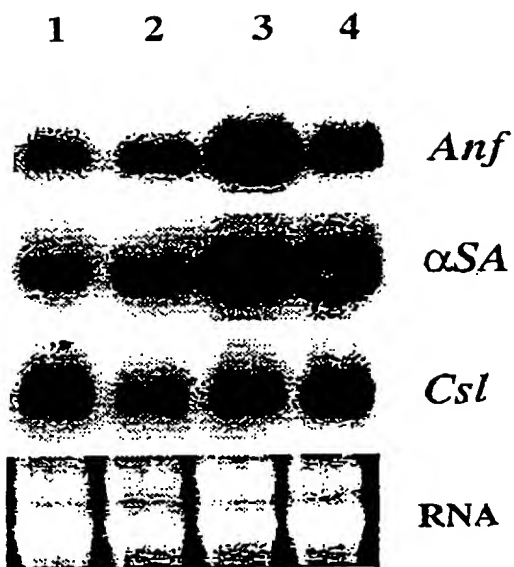


FIGURE 6

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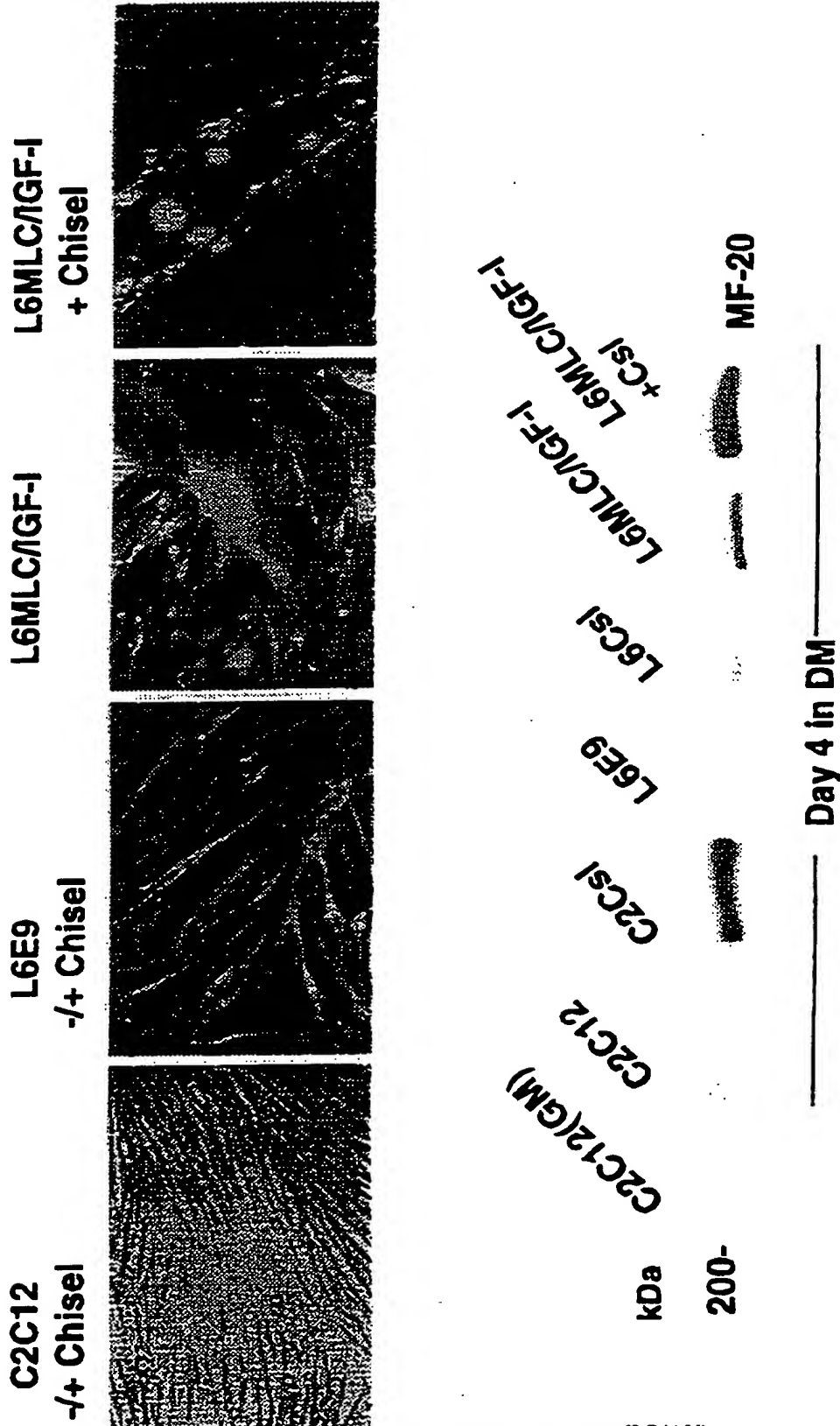


Figure 7

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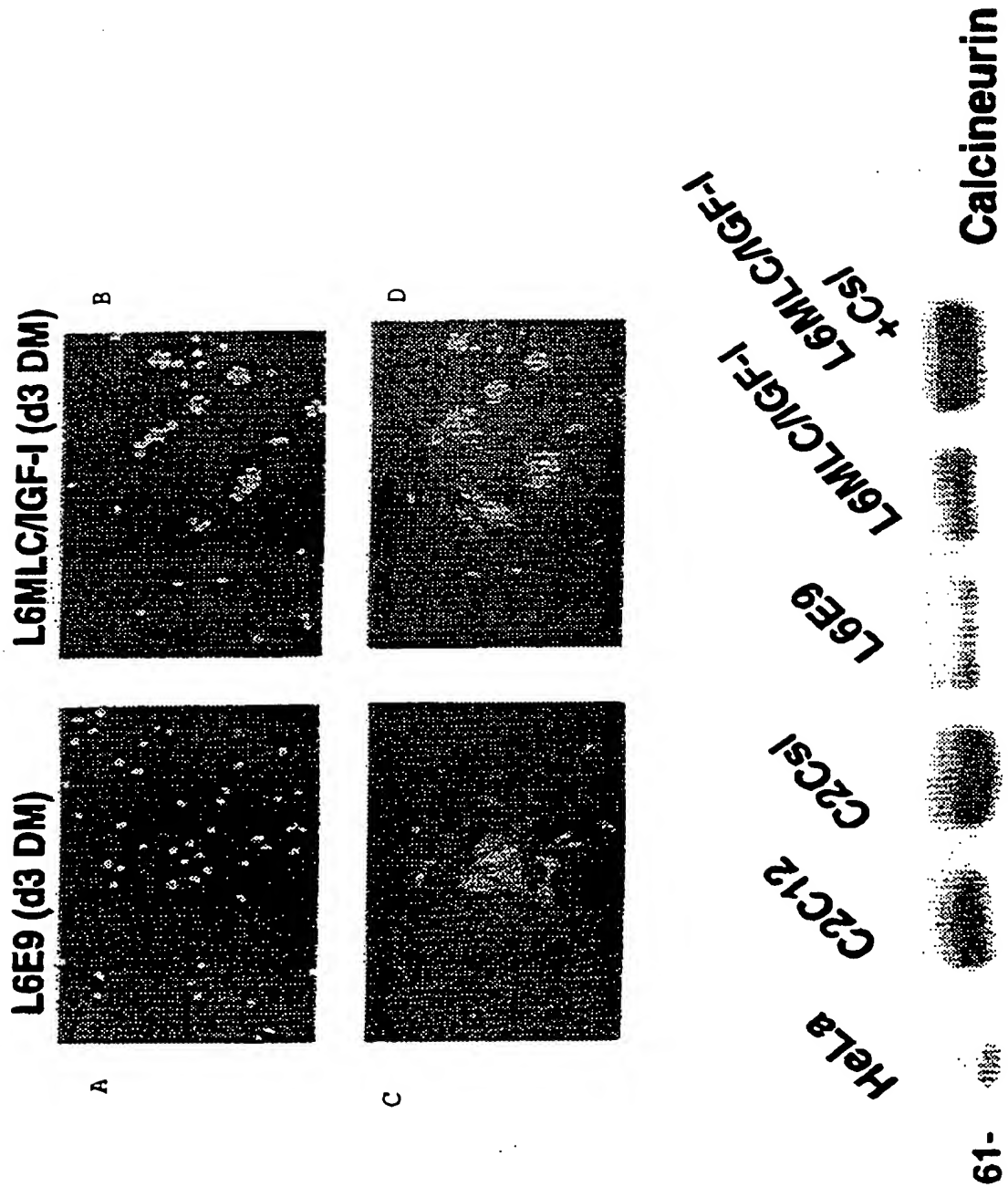


Figure 8

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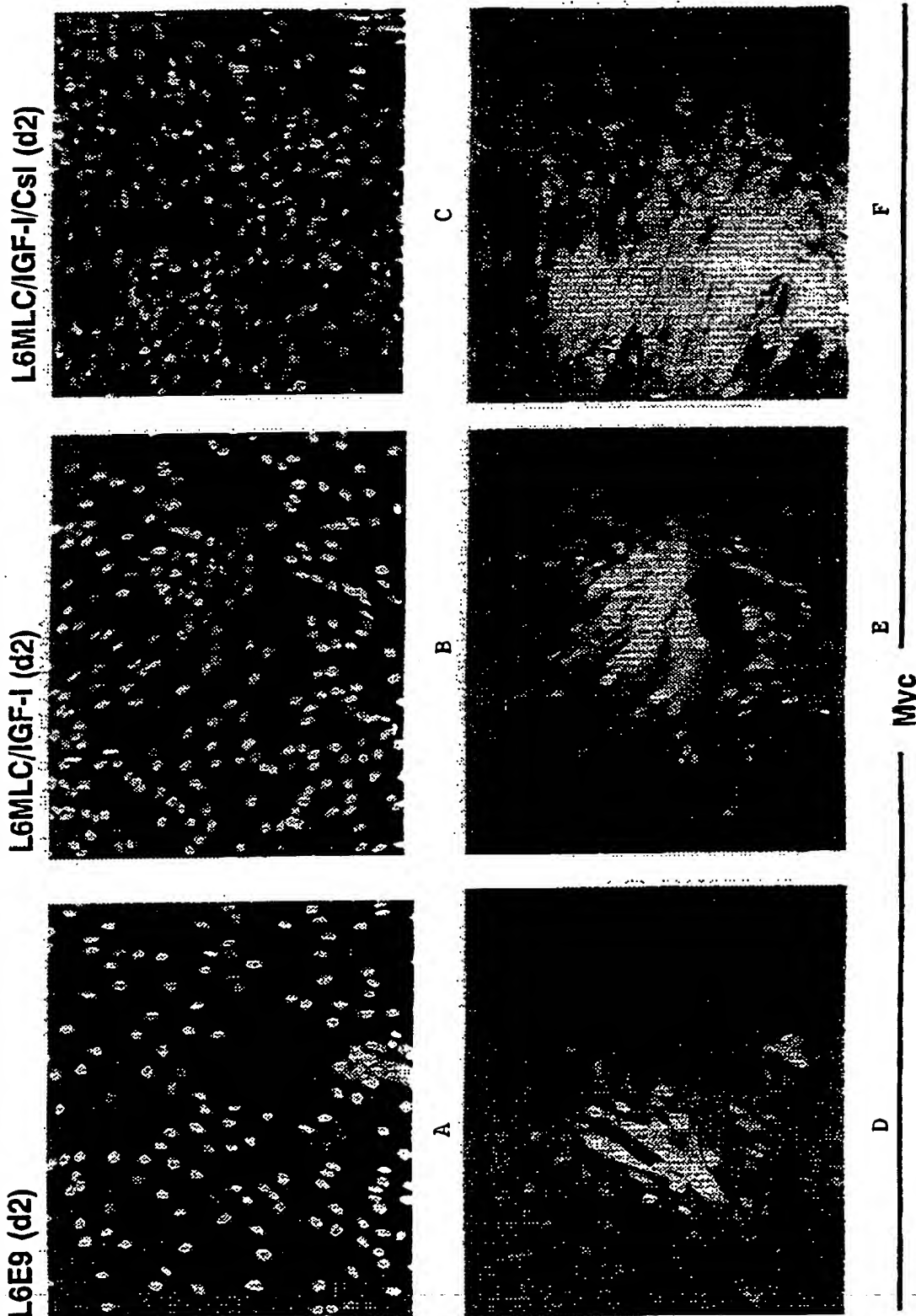


Figure 9

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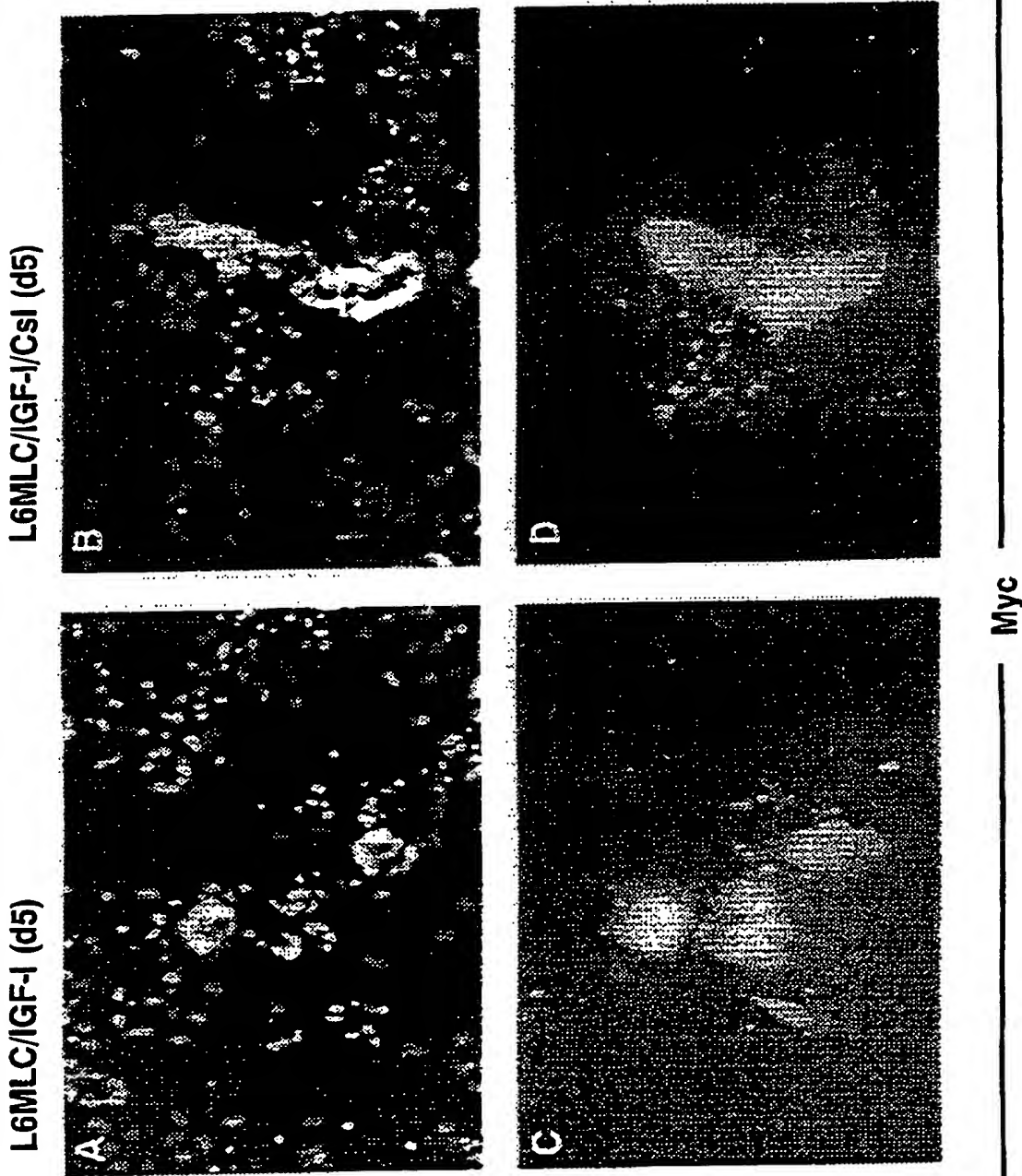


Figure 10

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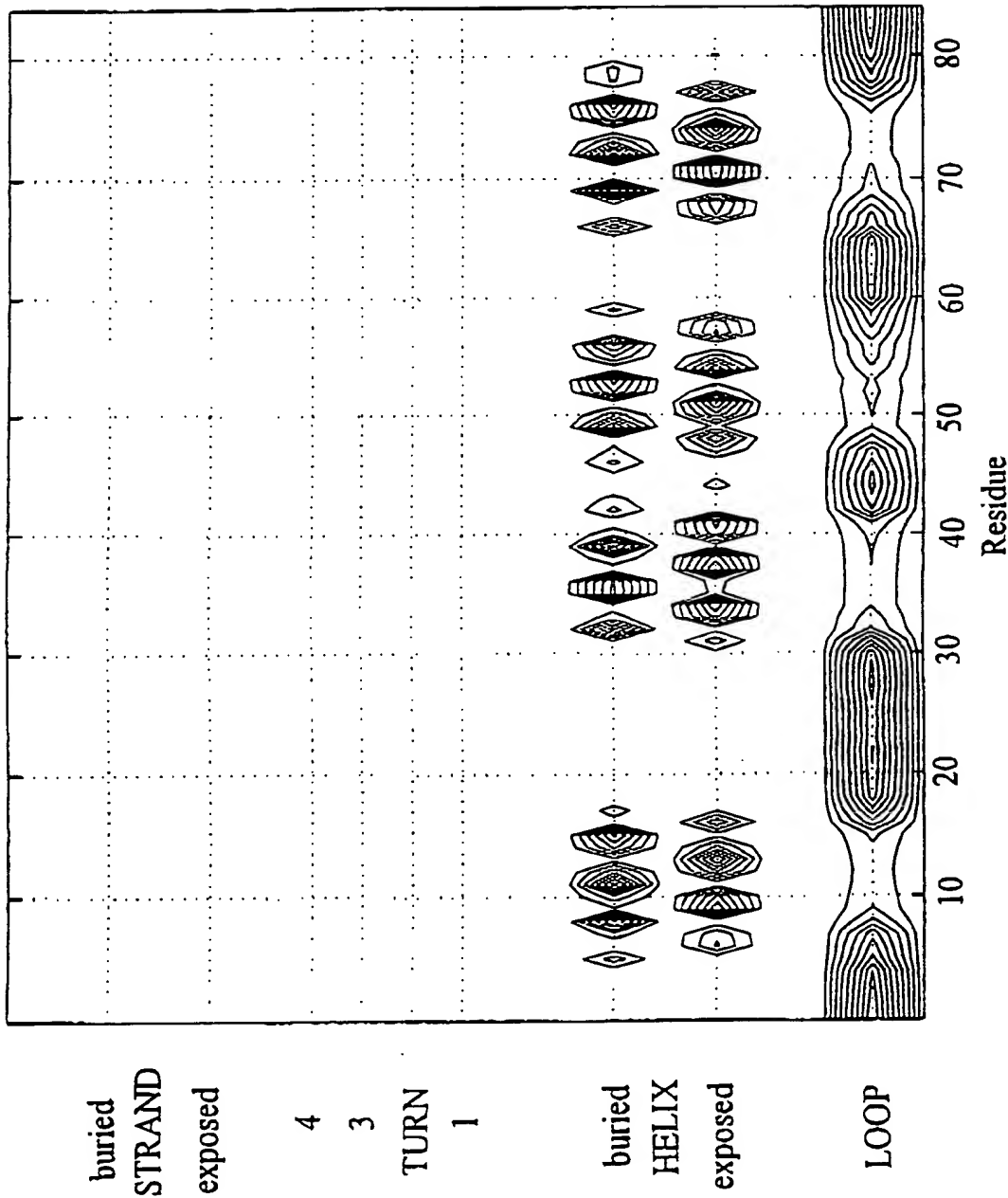


FIGURE 11

BMERC Protein Sequence Analysis System
<http://bmerc-www.bu.edu/psa/about.htm>

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Incomplete IQ Site

Binding to C-terminal lobe Binding to N-terminal lobe

N → C C ← N

Scallop MHC (R-MLC site): SVIQRNIRKwVLRlnwQwKLYSKVKPLLSIARQEEEMKE
 rat cardiac MHC (R-MLC site): LVIQWNIRAFMGVKWNWPMWKL YFKIKP LLSAETEKEMAN
 rat skeletal MHC (R-MLC site): FCIQYNIRAFMNVKHWPWMLFFKIKP LLSAETEKEMAT
 CALCINEURIN A (CnB site): ENNVNIRQFNCSPPHYWLPNFMDVFTWSL P FVGEKRVTE

CONSENSUS:

IQxxIR Wxxx^Yxx^V_FI

CALCINEURIN A mutations
 with reduced CnB binding:

A A

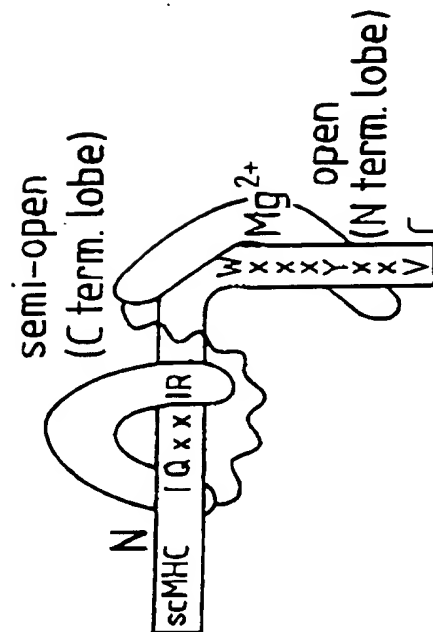


FIGURE 12

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Non-IQ Site

Binding to N-terminal lobe



Binding to C-terminal lobe



CaM Binding Sites

CALCINEURIN A: SEEDGFDGATAAARKEVIRWKIRAI GKMARVFSVLREESVS
SK MLCK (M13): KRRWKKNFIAVSAANRFKKISSGAL
Ca Pump (C24W): QILWFERGLNRIQTQIRVVNAFRSS
CALSPERMIN: ARRKLKAAVKAVVASSRLGS
MELLITIN: GIGAVLKVLTGGLPALISWIKRKRQQ

CaM

open (N term. lobe)

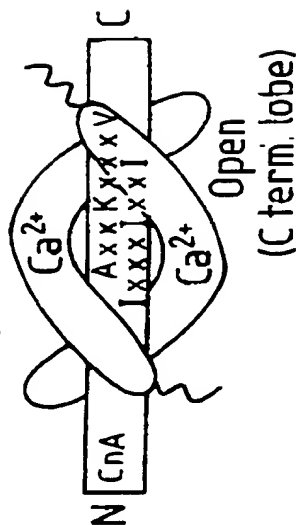


FIGURE 12

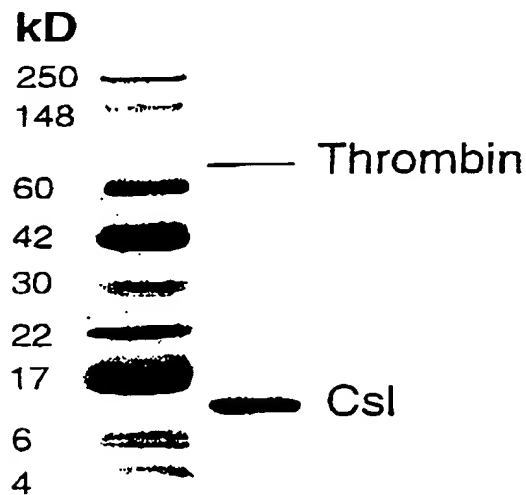
Alignments and binding modes taken from Houdusse and Cohen (1995) PNAS 92, 10644-10647 and Ikura et al. (1992) Science 256,632-638.
Calcineurin mutations found in Milan et al. (1994) Cell 79, 437-447.

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PAGE Analysis of Thrombin-Cleaved Bacterial GST-Chisel

**FIGURE 13**

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Western Blot Analysis of COS Cell-Expressed and Bacterial Chisel with Native Chisel Antibody

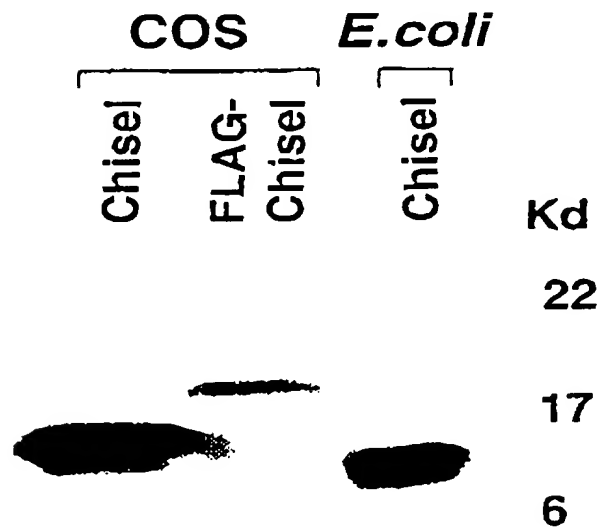


FIGURE 14

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MF-20

FLAG CHISEL

HOECHST



G



H



I



J



C



D



E



F

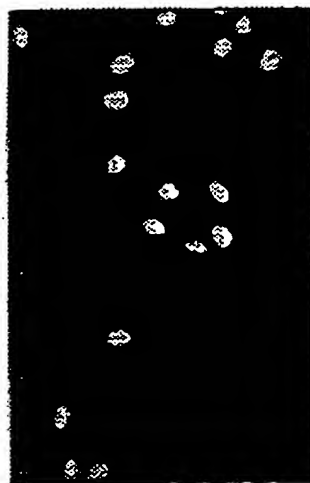


Figure 15

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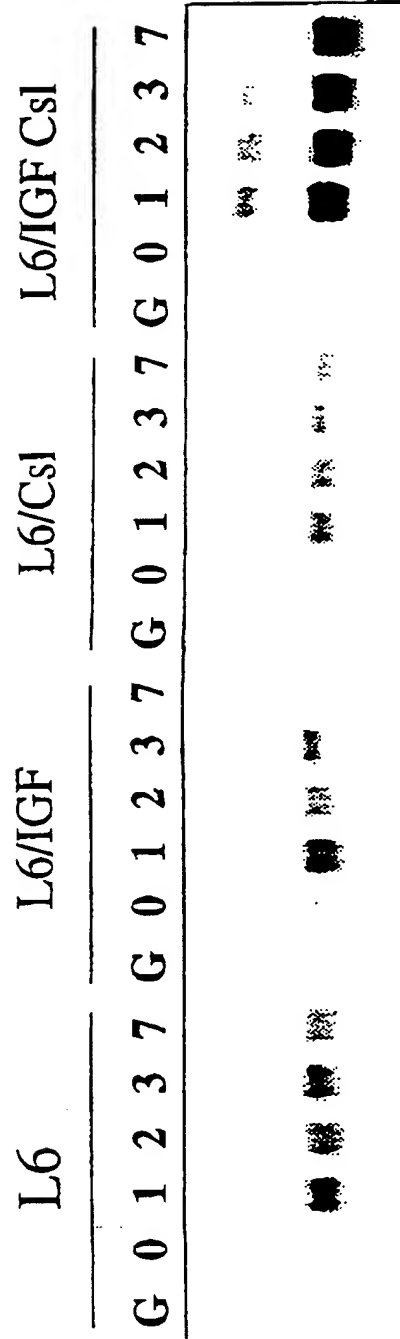


FIGURE 16

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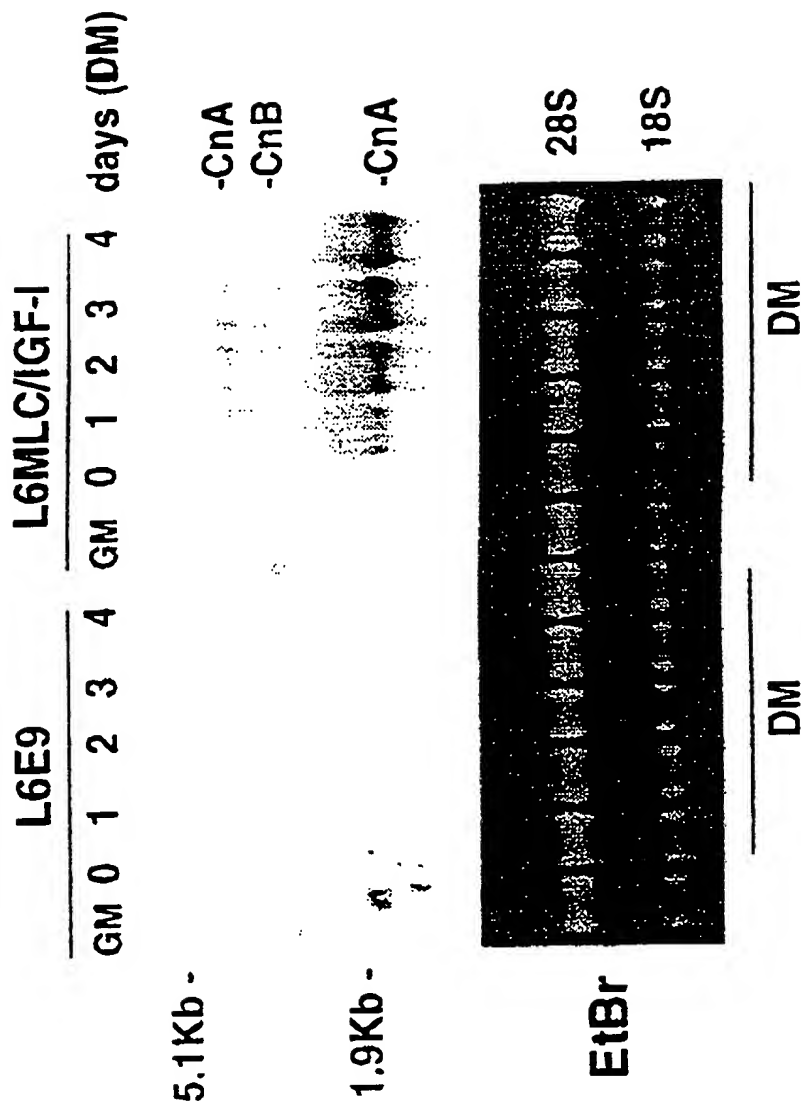


Figure 17 a

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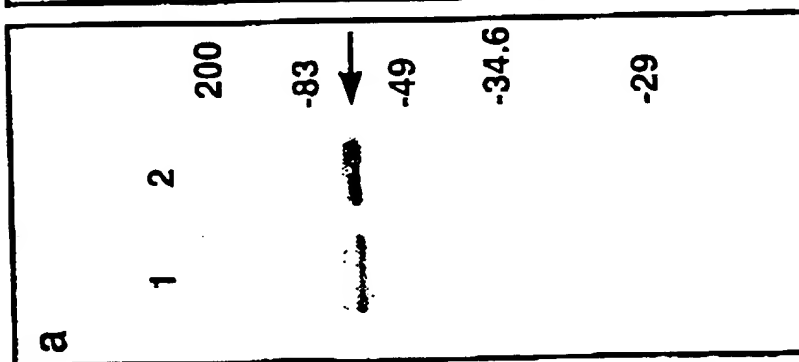
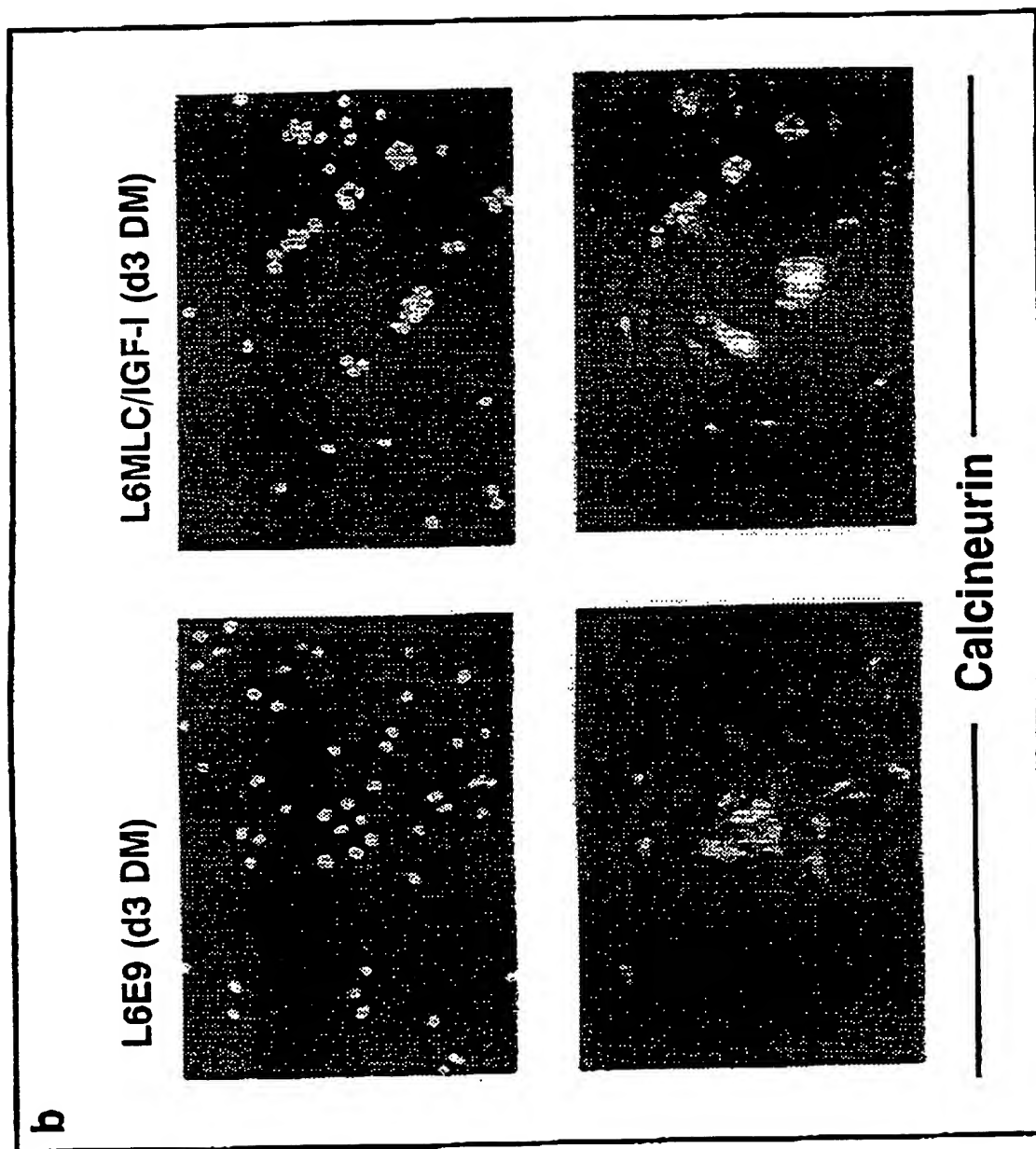


Figure 17b

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200 X

400 X

L6Myog/CnA (d2 DM)



L6E9 (d2 DM)

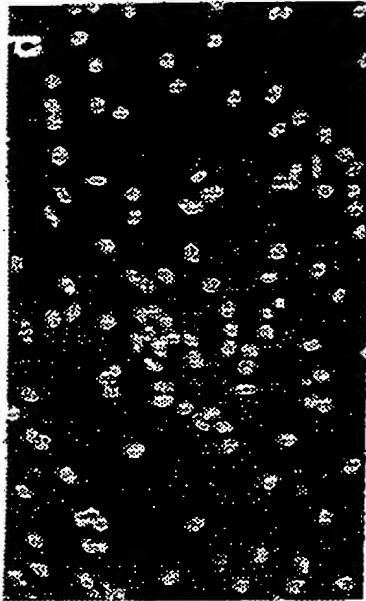


Figure 18a

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Hoechst



HA



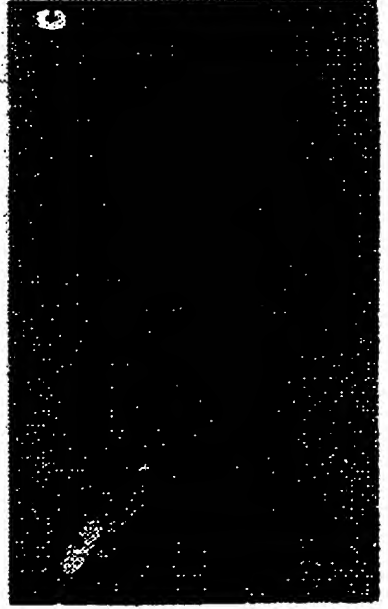
MF-20



Hoechst



HA



MF-20

Figure 18b

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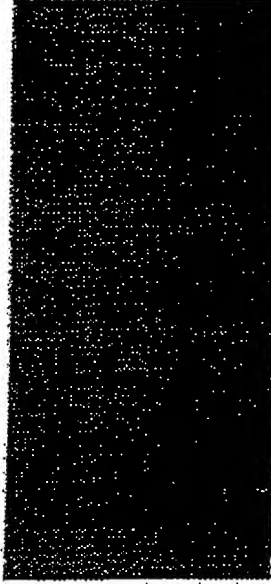
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L6MLC/GF-I - Untr. d5 DM



CsA d0 → d5 DM



CsA d2 → d5 DM



L6E9 - Untr. d5 DM



CsA d0 → d5 DM



CsA d2 → d5 DM

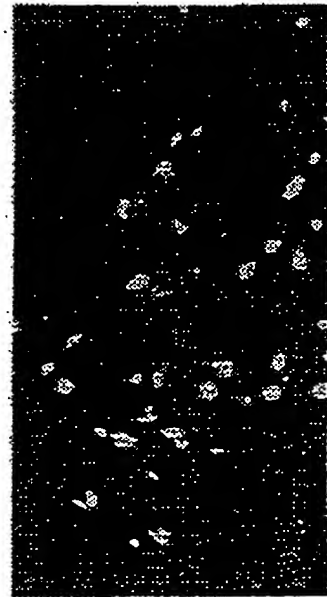


Figure 18c

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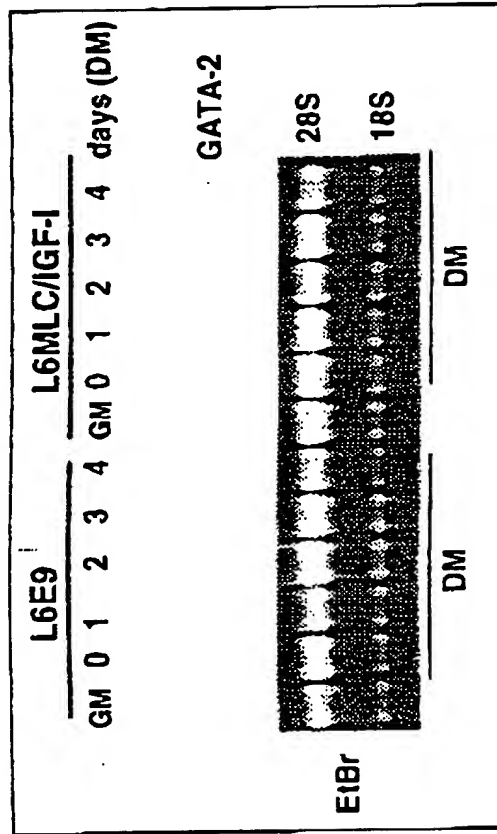


Figure 19a

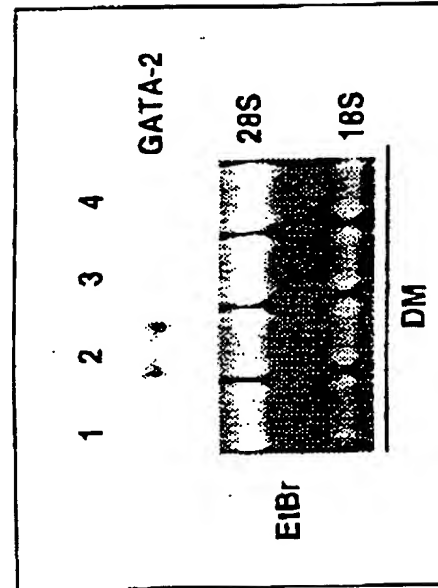


Figure 19b

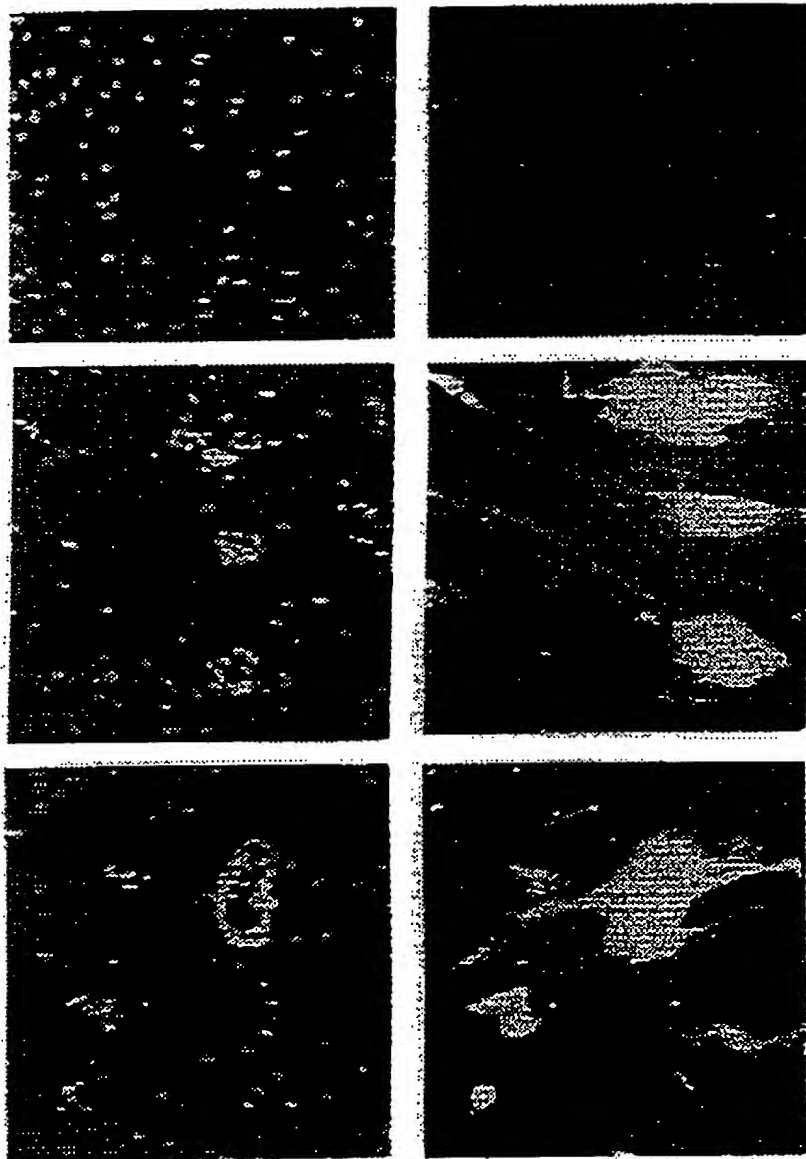
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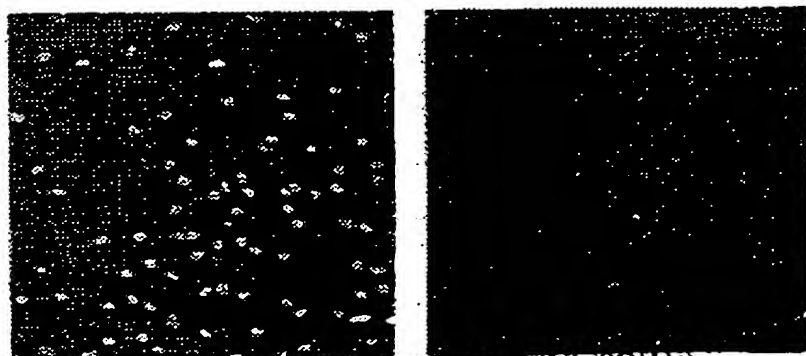
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FIG. 19c

L6MLC/IGF-1 (d3 DM)



L6E9 (d3 DM)



GATA-2

Figure 19c

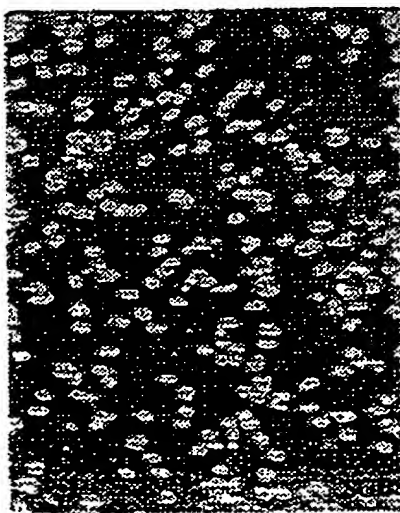
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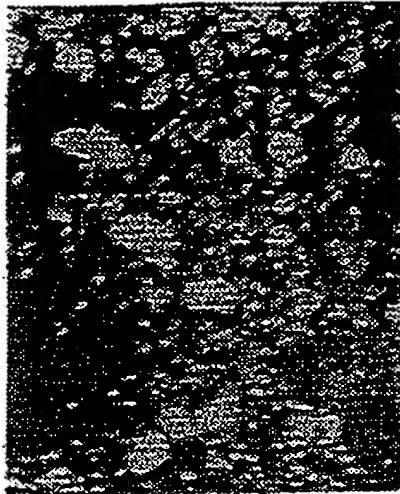
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FIGURE 19d

L6E9 (d3 DM)



L6Myog/CnA (d3 DM)



GATA-2

Figure 19d

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Control



IGF-1



IGF-1+ CsA

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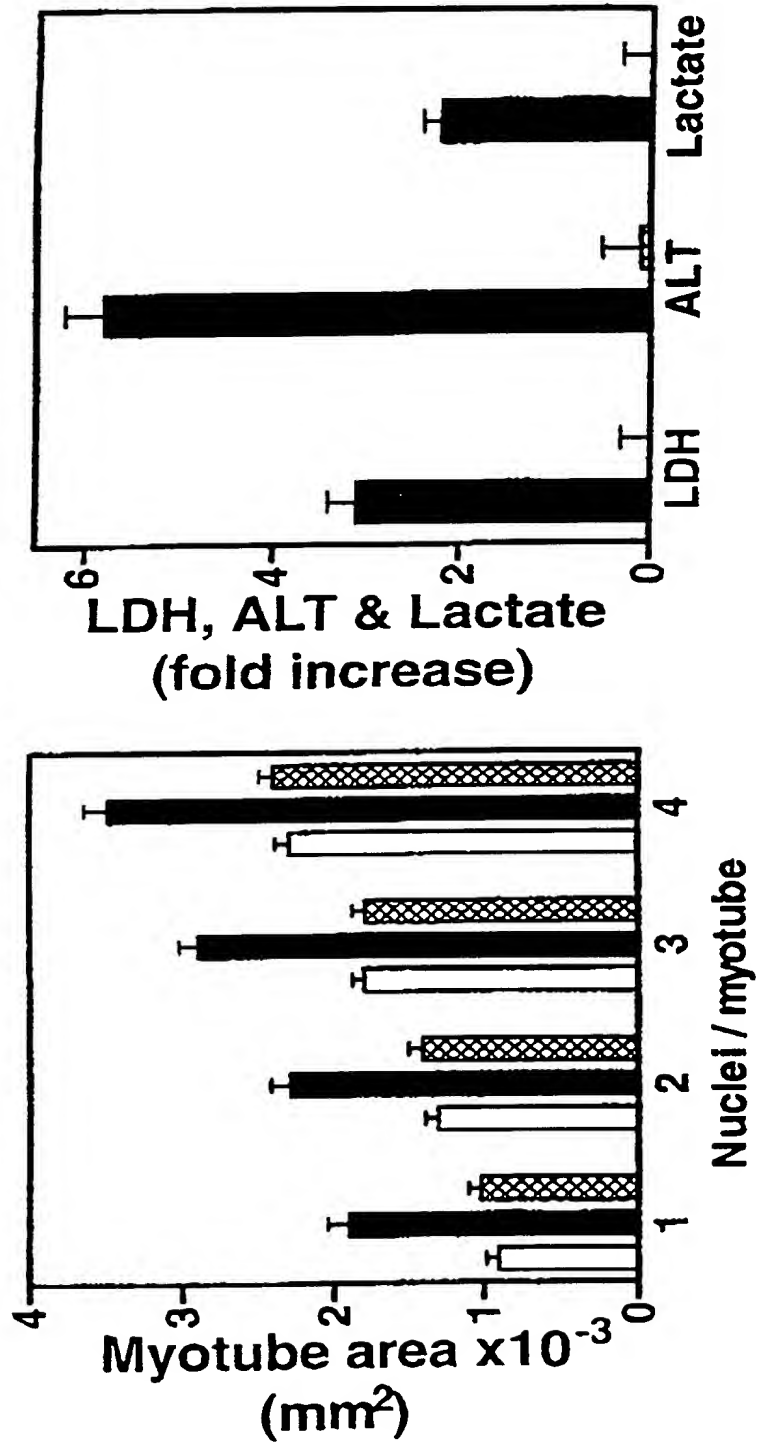


FIGURE 20B

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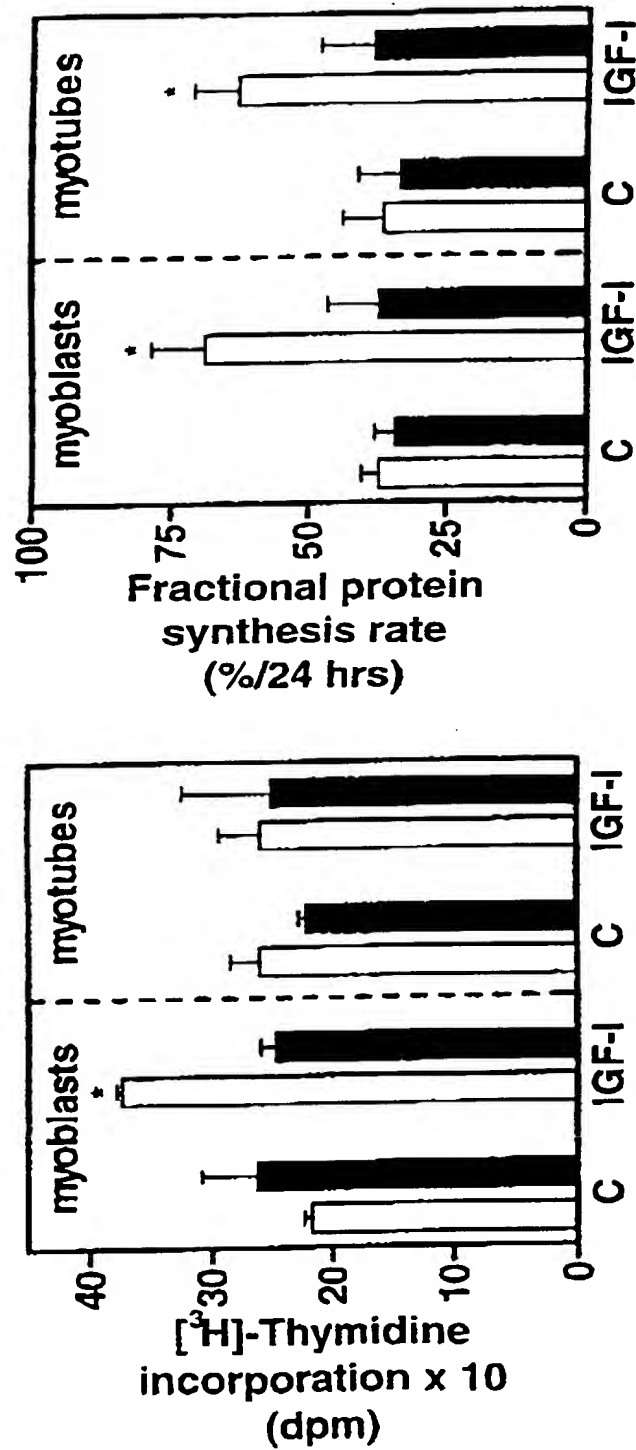


FIGURE 20C

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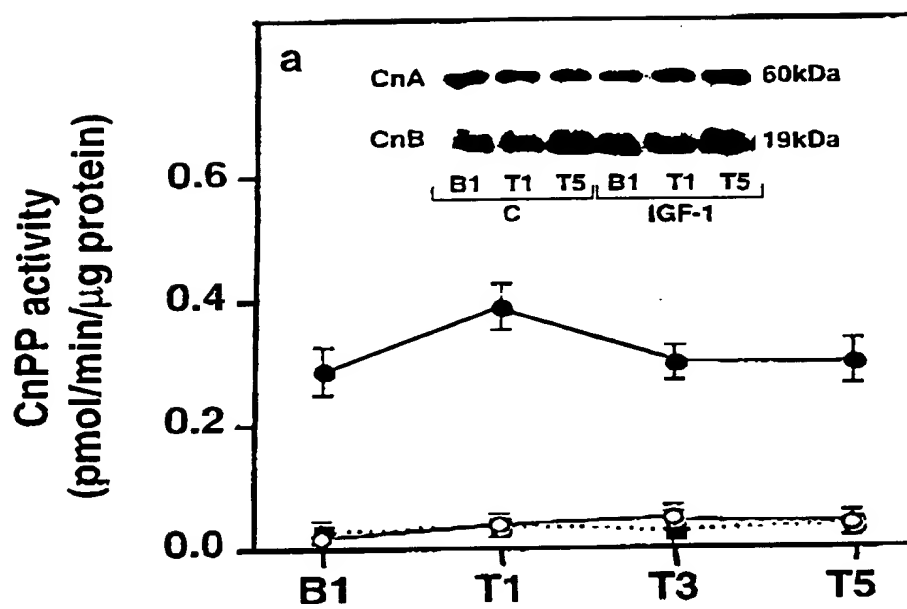


FIGURE 21

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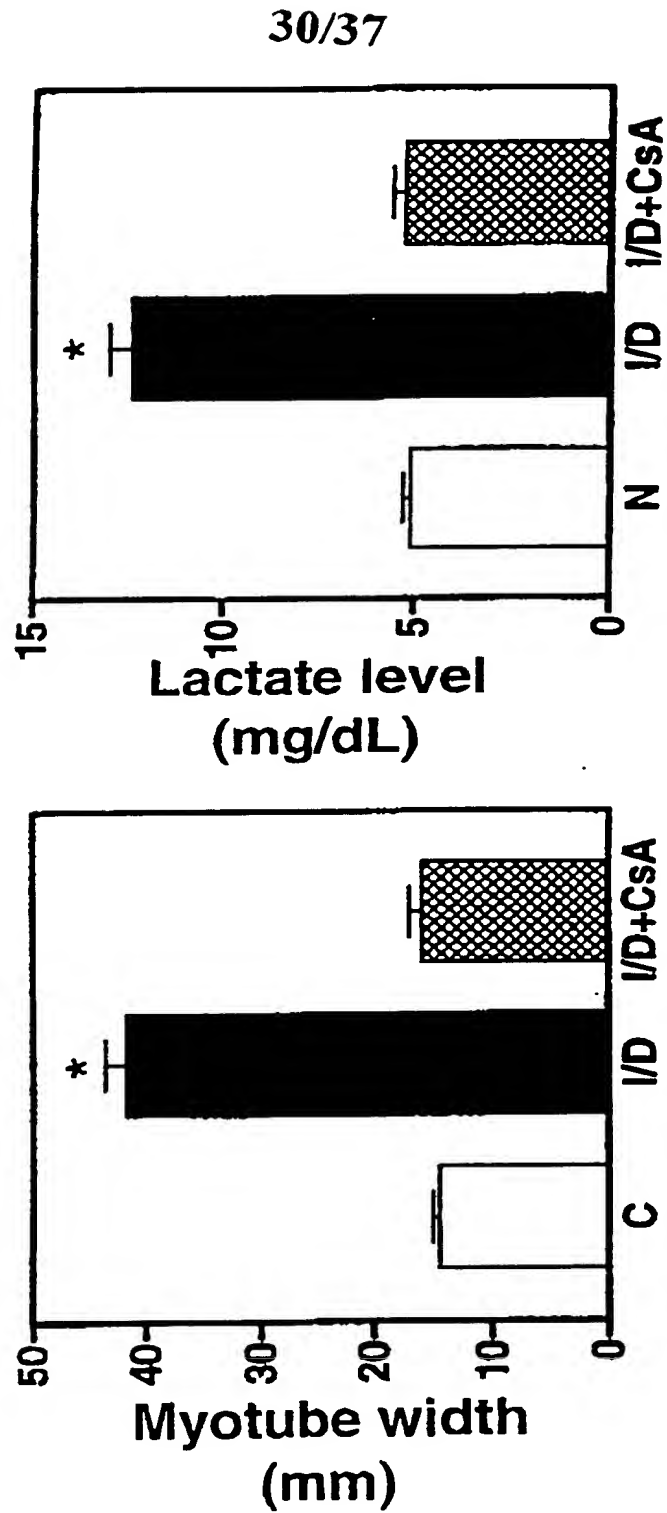


FIGURE 22A

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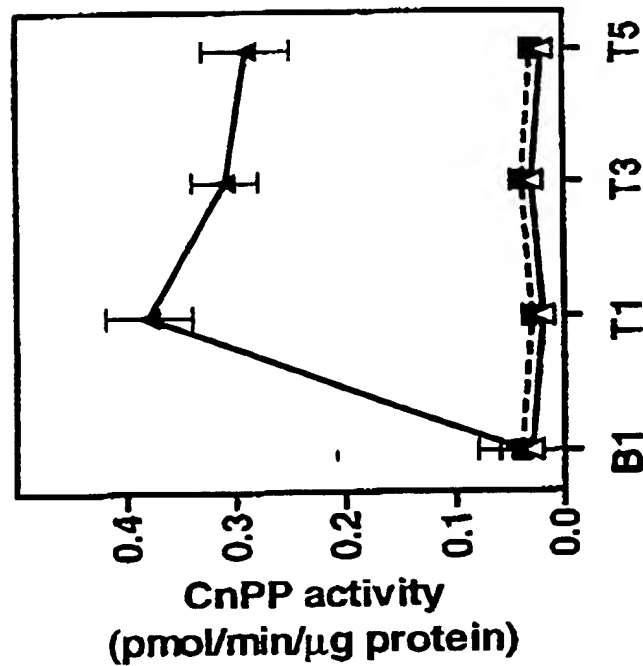
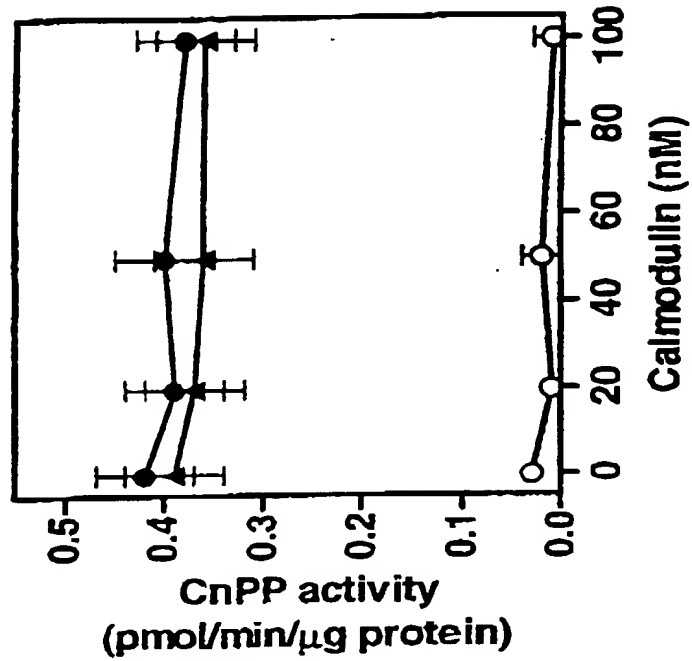


FIGURE 22B

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Figure 23 a

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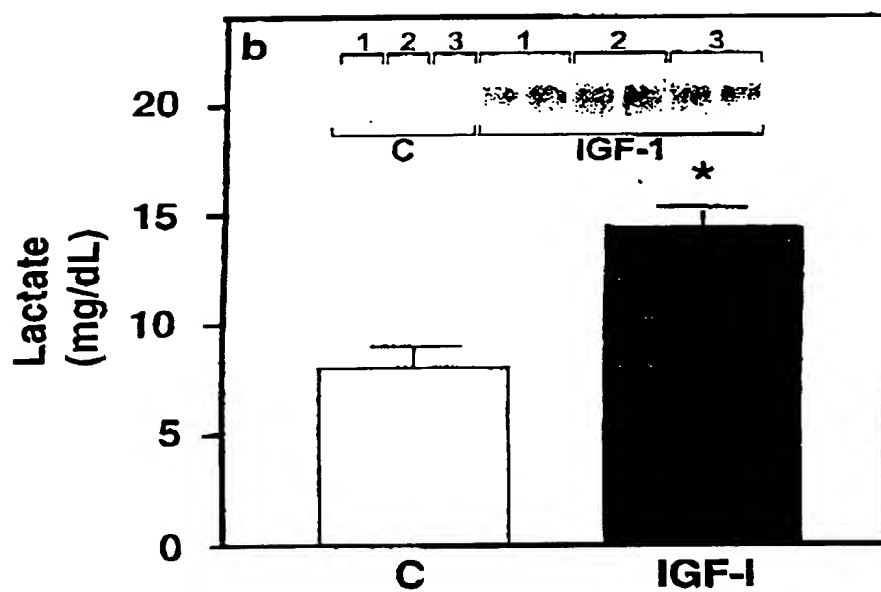


FIGURE 23B

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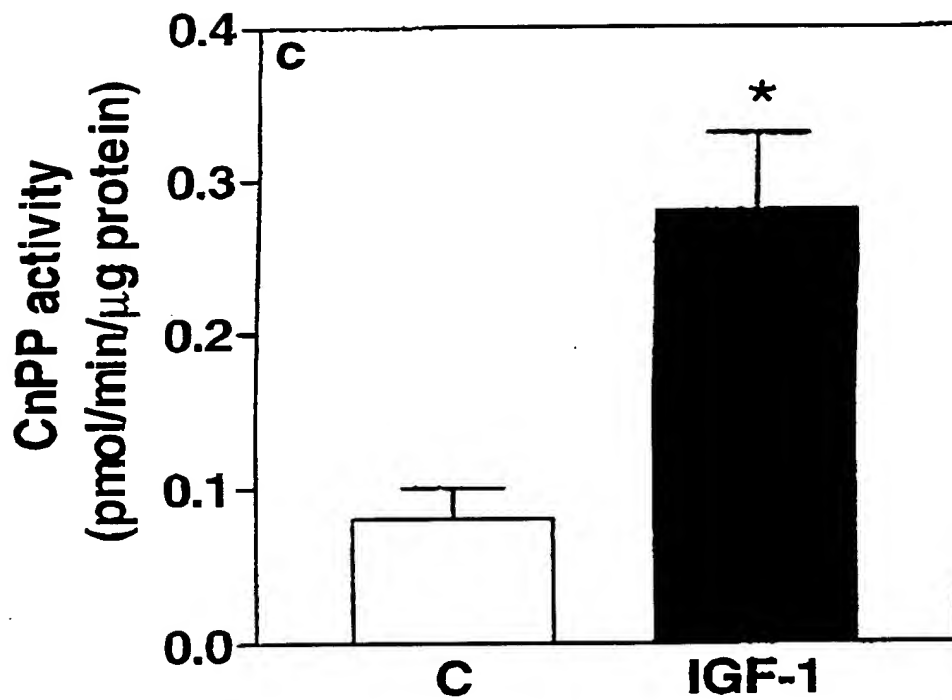
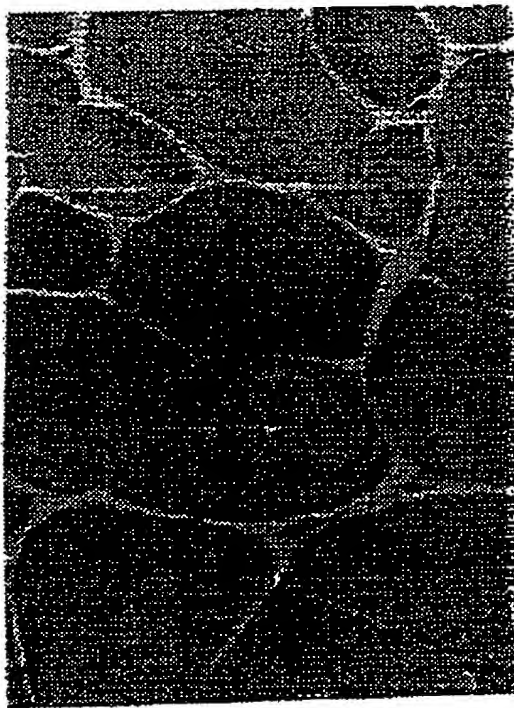


FIGURE 23C

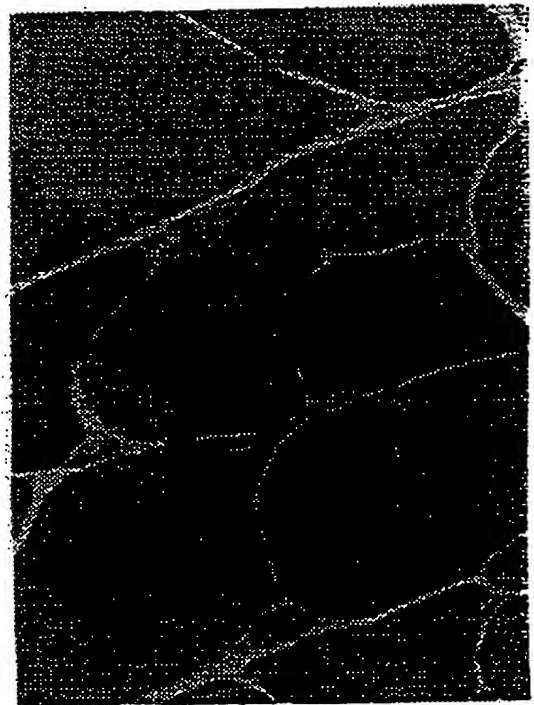
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Control



IGF-1

FIGURE 23D

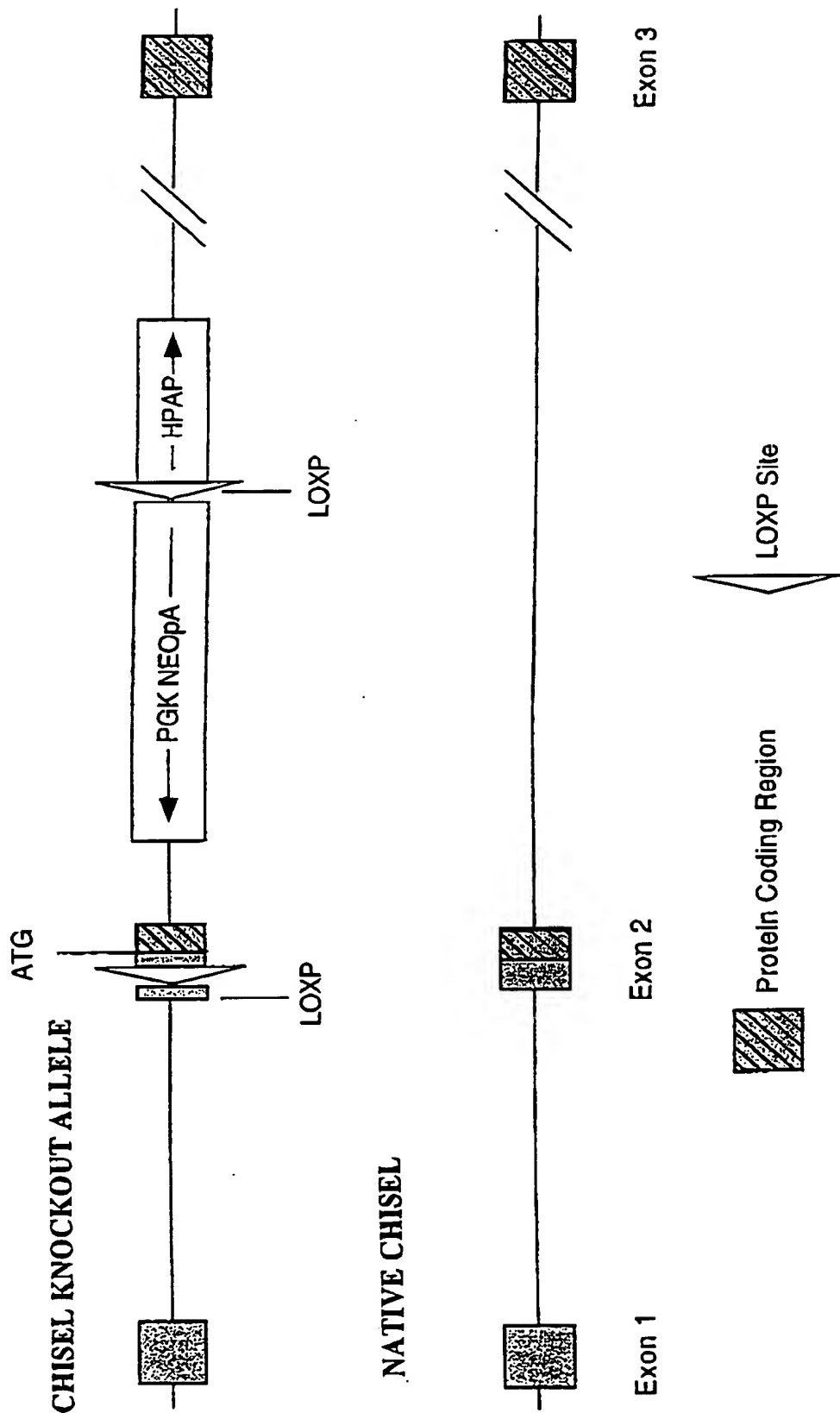
09/647019.042601

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FIGURE 24

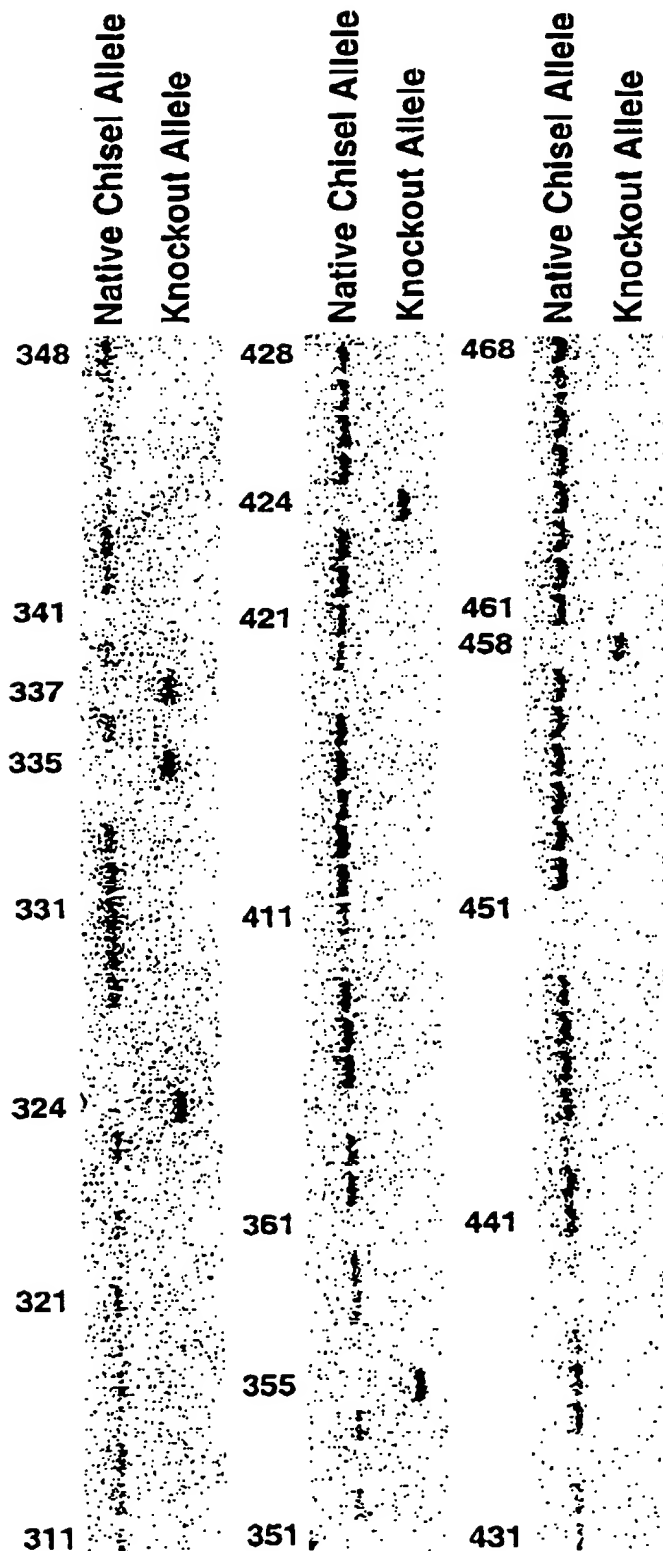


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Date: 17.11.98
 Filter: 10.11.98
 DNA: CSL311-468
 Digest: XbaI O/N
 Gel: 1%100V4hr
 Blot: Odep 2hr
 Probe: 2ul Csl on 10.11.98
 Incorp: 3.9dpm exp7
 Prehyb: >15' @ 42C
 Hyb: O/N @ 42C
 Wash: X3X1 @ 68C>1hr
 PI: 4d

FIGURE 25